

COBAS AmpliScreen™ HIV-1 Test, v1.5

Package Insert

Table of Contents

Table of Contents	ii
Intended Use	1
Summary and Explanation of the Test	2
Principles of the Procedure	3
Sample Processing	3
Reverse Transcription	4
PCR Amplification	4
Selective Amplification	5
Hybridization Reaction	5
Detection Reaction	6
Materials Provided by Roche	6
Other Materials Required But Sold Separately (May Be Purchased from Roche)	8
Materials Required But Not Provided by Roche	8
Reagents	9
COBAS AmpliScreen Multiprep Specimen Preparation and Control Kit, 96 Tests	9
COBAS AmpliScreen HIV-1 Test, version 1.5, 96 Tests	10
COBAS AMPLICOR Wash Buffer, 500 Tests	12
Storage Instructions	13
Precautions	13
Reagent Preparation	16
Specimen Collection, Storage and Pooling	19
Procedural Notes	21
Instructions for Use	25
Amplification, Hybridization and Detection of Stored Processed Specimens	25
Hybridization and Detection of Stored Denatured Amplicon	25
A. Reagent Preparation — Working Master Mix	26
B. Specimen and Control Preparation	26
C. Reverse Transcription, Amplification and Detection	32

Quality Control Procedures	34
Interpretation of Results	36
Procedural Limitations	39
Performance Characteristics	40
Reproducibility	40
Analytical Sensitivity — Dilutional Panels	42
Analytical Sensitivity — WHO HIV-1 International Standard	43
Analytical Sensitivity — CBER HIV-1 Panel.....	45
Group / Subtype Detectability.....	45
Seroconversion Panels	46
Dilutional Sensitivity with Weakly Reactive HIV-1 p24 Antigen Samples.....	48
Dilutional Sensitivity with Weakly Reactive HIV-1 Antibody Positive Samples	48
Analytical Specificity — Potentially Cross Reactive and Interfering Microorganisms	49
Analytical Specificity — Non-HIV-1 Samples.....	50
Potentially Interfering Substances.....	50
Endogenous Interfering Substances.....	50
Exogenous Interfering Substances.....	50
Clinical Performance	51
AIDS and HIV-1 Asymptomatic Populations	51
High Risk Population.....	52
Pool Reactivity in Volunteer Blood Donors	54
Detection of Window Period Cases.....	55
Performance of Single Donation Testing	56
References	57

COBAS AmpliScreen HIV-1 Test, v1.5 (96 Test)

COBAS AmpliScreen HIV-1 Test, version1.5	96 Tests	P/N: 3322114018
COBAS AmpliScreen Multiprep Specimen Preparation and Control Kit	96 Tests	P/N: 3302555018
COBAS AMPLICOR™ Wash Buffer	500 Tests	P/N: 20759899123 ART: 07 5989 9 US: 83314

Intended Use

The COBAS AmpliScreen HIV-1 Test, version 1.5 (v1.5) is a qualitative *in vitro* test for the direct detection of Human Immunodeficiency Virus (HIV-1) RNA in human plasma from donations of whole blood and blood components for transfusion.

The COBAS AmpliScreen HIV-1 Test, v1.5 is intended for use in screening of individual donor samples of human plasma, or pools of human plasma comprised of equal aliquots of not more than 24 individual donations. The COBAS AmpliScreen HIV-1 Test, v1.5 is intended to be used for detecting HIV-1 RNA in conjunction with licensed tests for detecting antibodies to HIV-1.

This assay may be used as an alternative to licensed HIV-1 p24 antigen tests for screening human plasma from donations of whole blood and blood components.

This assay is not intended for use as an aid in diagnosis.

Summary and Explanation of the Test

Human Immunodeficiency Virus (HIV-1) is the etiologic agent of Acquired Immunodeficiency Syndrome (AIDS)¹⁻³. HIV-1 infection can be transmitted by sexual contact, exposure to infected blood or blood products, or by an infected mother to the fetus⁴. Within three to six weeks of exposure to HIV-1, infected individuals generally develop a brief, acute syndrome characterized by flu-like symptoms and associated with high levels of viremia in the peripheral blood⁵⁻⁸. In most infected individuals this is followed by an HIV-1-specific immune response and a decline of plasma viremia, usually within four to six weeks of the onset of symptoms^{9,10}. The prevalence of HIV-1 infection is 1.1% overall in the world, 0.56% in North America and 0.25% in West Europe¹¹.

Serological screening assays have greatly reduced, but not completely eliminated, the risk of transmitting viral infections by transfusion of blood products¹²⁻¹⁵. HIV-1 p24 antigen is the principal core protein of HIV-1 and is found in serum or plasma either free or bound by anti-p24 antibody. HIV-1 p24 antigen can be measured with commercially available enzyme immunoassays (EIA), which reduce the seroconversion window period, i.e., the time between infection and the rise of antibodies to the virus¹⁶, by approximately 5 to 6 days^{17,18}. Recent studies indicate that nucleic acid based amplification tests for HIV-1 RNA will further reduce the residual transmission risk by detecting HIV-1 RNA in donations made during the seroconversion window period. Nucleic acid-based tests can detect viremic units donated by carriers who do not seroconvert or who lack antibodies to serological markers normally detected by immunological assays^{16,19,20}.

HIV-1 RNA in plasma can be detected by nucleic acid amplification technologies, such as the PCR²¹⁻²³. The COBAS AmpliScreen HIV-1 Test, v1.5 uses PCR technology to achieve maximum sensitivity for the detection of HIV-1 RNA in plasma samples²⁴.

A number of proposals have been made for performing nucleic acid tests on mini-pools comprised of small aliquots from many individual samples. The high sensitivity of PCR

has demonstrated that potentially infectious donations contained within mini-pools can be detected even if the mini-pool contains a single viremic donor.^{13,25,26}

The assay incorporates an Internal Control for monitoring assay performance in each individual test as well as AmpErase® to reduce potential contamination by previously amplified material (amplicon).

Principles of the Procedure

The COBAS AmpliScreen HIV-1 Test, v1.5 is based on five major processes:

1. Sample Processing
2. Reverse transcription of target RNA to generate complementary DNA (cDNA)²⁷
3. PCR amplification²⁷ of target cDNA using HIV-specific complementary primers
4. Hybridization of the amplified products to oligonucleotide probes specific to the target(s)
5. Detection of the probe-bound amplified products by colorimetric determination.

Sample Processing

Two specimen processing procedures are used with the COBAS AmpliScreen HIV-1 Test, v1.5 as follows:

- Multiprep Specimen Processing Procedure for preparation of mini-pool specimens
- Standard Sample Processing for preparation of individual donor samples

In the Standard Specimen Processing Procedure, HIV-1 RNA is isolated directly from plasma by lysis of the virus particles with Multiprep Lysis Reagent followed by precipitation of the RNA with alcohol. In the Multiprep Specimen Processing Procedure, HIV-1 viral particles are first pelleted from the plasma sample by high speed

centrifugation, followed by lysis of the pelleted virus with a chaotropic agent (Multiprep Lysis Reagent) and precipitation of the RNA with alcohol.

The Multiprep Internal Control (MP IC), containing the HIV-1 Internal Control, is introduced into each sample with the Multiprep Lysis Reagent and serves as an extraction and amplification control for each processed specimen and control. The HIV-1 Internal Control is an RNA transcript with primer binding regions identical to those of the HIV-1 target sequence, a randomized internal sequence of similar length and base composition as the HIV-1 target sequence, and a unique probe binding region that differentiates the HIV-1 Internal Control amplicon from target amplicon. These features were selected to ensure equivalent amplification of the HIV-1 Internal Control and the HIV-1 target RNA.

Reverse Transcription

The reverse transcription and amplification reactions are performed with the thermostable recombinant enzyme *Thermus thermophilus* DNA Polymerase (*rTth* pol). In the presence of manganese (Mn^{2+}) and under the appropriate buffer conditions, *rTth* pol has both reverse transcriptase and DNA polymerase activity.²⁸ This allows both reverse transcription and PCR amplification to occur in the same reaction mixture. Reverse transcription using *rTth* pol produces a cDNA copy of the HIV-1 target and the HIV-1 Internal Control RNA.

PCR Amplification

Following reverse transcription using *rTth* pol, a second DNA strand is produced from the cDNA copy, thereby yielding a double-stranded DNA copy of the HIV-1 target and HIV-1 Internal Control RNA. The reaction mixture is heated to separate the resulting double-stranded DNA. As the mixture cools, primers anneal to the target DNA, in the presence of Mn^{2+} and excess deoxynucleotide triphosphates (dNTPs), extends the annealed primers along the target templates to produce a double-stranded DNA molecule termed an amplicon. The COBAS AMPLICOR Analyzer automatically repeats this

process for a designated number of cycles, each cycle effectively doubling the amount of amplicon DNA. The required number of cycles is preprogrammed in the COBAS AMPLICOR Analyzer.

Selective Amplification

To ensure selective amplification of nucleic acid target in the sample and prevent amplification of pre-existing amplicon, AmpErase® (uracil-N-glycosylase, UNG) is added to the COBAS AmpliScreen HIV-1 Test, v1.5. AmpErase recognizes and catalyzes the destruction of DNA strands containing deoxyuridine²⁹, but not DNA containing deoxythymidine. Deoxyuridine is not present in naturally occurring DNA, but is always present in amplicon because of the use of deoxyuridine triphosphate in place of deoxythymidine triphosphate as one of the dNTPs in the Master Mix reagent; therefore, only amplicon contain deoxyuridine. Deoxyuridine renders contaminating amplicon susceptible to destruction by AmpErase before amplification of the target DNA. AmpErase, which is included in the Master Mix reagent, catalyzes the cleavage of DNA, thereby rendering the DNA non-amplifiable. AmpErase is inactive at temperatures above 55°C, i.e., throughout the thermal cycling steps, and therefore does not destroy target amplicon. Following amplification, any residual enzyme is denatured by the addition of the denaturation solution, thereby preventing the degradation of any target amplicon.

Hybridization Reaction

Following PCR amplification, the COBAS AMPLICOR Analyzer automatically adds denaturation solution to the A-tubes to chemically denature the HIV-1 target amplicon and the HIV-1 Internal Control amplicon to form single-stranded DNA. Aliquots of denatured amplicon are then transferred to two detection cups (D-cups). A suspension of magnetic particles coated with an oligonucleotide probe specific for HIV-1 target amplicon or HIV-1 Internal Control amplicon is added to the individual D-cups. The biotin-labeled HIV-1 target and HIV-1 Internal Control amplicon are hybridized to the target-specific oligonucleotide probes bound to the magnetic particles. This hybridization

of amplicon to the target-specific probe increases the overall specificity of the COBAS AmpliScreen HIV-1 Test, v1.5.

Detection Reaction

Following the hybridization reaction, the COBAS AMPLICOR Analyzer washes the magnetic particles in the D-cups to remove unbound material, and then adds Avidin-Horseradish Peroxidase Conjugate. The Avidin-Horseradish Peroxidase Conjugate binds to the hybridized biotin-labeled amplicon. The COBAS AMPLICOR Analyzer removes unbound conjugate by washing the magnetic particles and then adds a substrate solution containing hydrogen peroxide and 3,3',5,5'-tetramethylbenzidine (TMB) to each D-cup. In the presence of hydrogen peroxide, the particle-bound horseradish peroxidase catalyzes the oxidation of TMB to form a colored complex. The absorbance is measured by the COBAS AMPLICOR Analyzer at a wavelength of 660 nm.

Materials Provided by Roche

The COBAS AmpliScreen Multiprep Specimen Preparation and Control Kit and the COBAS AMPLICOR Wash Buffer kit are provided as stand-alone kits to be used in conjunction with the COBAS AmpliScreen HIV-1 Test, v1.5, as well as the COBAS AmpliScreen HCV Test, v2.0, and the COBAS AmpliScreen HBV Test.

COBAS AmpliScreen Multiprep Specimen Preparation and Control Kit (96 tests) (P/N: 3302555018)

MP (+) C	(Multiprep Positive (+) Control)
MP LYS	(Multiprep Lysis Reagent)
MP DIL	(Multiprep Specimen Diluent)
MP IC	(Multiprep Internal Control)
MP (-) C	(Multiprep Negative (-) Control)
NHP	(Negative Plasma (Human))

COBAS AmpliScreen HIV-1 Test, v1.5 (96 tests)
(P/N: 3322114018)

COBAS AmpliScreen HIV-1 Amplification Reagents, version 1.5

HIV-1 MMX, v1.5 (HIV-1 Master Mix, version 1.5)
HIV-1 Mn²⁺, v1.5 (HIV-1 Manganese Solution, version 1.5)

COBAS AmpliScreen HIV-1 Detection Reagents, version 1.5

DN4 (Denaturation Solution)
IH PS1, v1.5 (HIV-1 Probe Suspension 1, version 1.5)
IH4, v1.5 (HIV-1 Probe Suspension 2, version 1.5)
II PS1 (IC Probe Suspension 1)
II4 (IC Probe Suspension 2)
CN4 (Avidin - Horseradish Peroxidase Conjugate)
SB3 (Substrate A)
SB (Substrate B)

COBAS AMPLICOR Wash Buffer Kit (500 tests)
(P/N: 20759899123; ART: 07 5989 9; US: 83314)

WB (10X-Wash Concentrate)

Other Materials Required But Sold Separately (May Be Purchased from Roche)

- COBAS AMPLICOR Analyzer with software version 0022B, Printer and *Operator's Manual*
- COBAS AMPLICOR A-rings
- COBAS AMPLICOR D-cups
- AMPLILINK™ Software version 1.3 and *Operator's Manual*
- Hamilton MICROLAB AT plus 2 Pipettor, the COBAS AmpliScreen Pooling System Guide, Software Kit (Hamilton SUNPLUS Data Station and RUNENDE Software, and the Roche Pooling Methods Software version 1.4)
- Sarstedt 1.5-mL tube Barcode Labels
- Hamilton Archive and Intermediate Plate Barcode Labels
- Refrigerated high speed centrifuge with fixed angle rotor (45 degrees, capacity for at least 24 x 1.5-mL tubes) with an RCF of 23,600 x g (Heraeus Centrifuge 17RS or Biofuge 28RS with HFA 22.1 rotor, Heraeus Biofuge Stratos with the 3331 rotor or equivalent).

Materials Required But Not Provided by Roche

- Microcentrifuge, (max. RCF 16,000 x g, min. RCF 12,500 x g) (Eppendorf 5415C, HERMLE Z230M, or equivalent)
- Eppendorf® 1.25 mL Combitip® Reservoir (sterile) or equivalent
- Eppendorf Multipipette® pipette or equivalent
- Ethanol, 90% or 95%, reagent grade for Molecular Biology or Histology use
- Distilled or deionized water
- Powderless disposable gloves
- Isopropyl alcohol, reagent grade

- Disposable, Sterile, Polystyrene Pipettes(5 mL, 10 mL and 25 mL)
- Sterile, RNase-free, fine-tip transfer pipettes,
- Pipettors (capacity 20 µL to 1000 µL capable of providing ± 3% accuracy and precision ≤ 5%) with aerosol barrier or positive displacement RNase-free tips
- Tube racks (Sarstedt P/N 93.1428 or equivalent)
- 1.5 mL sterile, non-siliconized, conical polypropylene screw-cap tubes, , (Sarstedt 72.692.105 or equivalent)
- Vortex mixer
- Hamilton Slotted Deepwell Archive Plate, 2.2 mL and Sealing Capmat
- Hamilton Slotted Intermediate Plate

Reagents

COBAS AmpliScreen Multiprep Specimen Preparation and Control Kit, 96 Tests

MP (+) C (Multiprep Positive (+) Control) 8 x 0.1 mL
Tris-HCl buffered solution containing noninfectious RNA transcripts for HCV and HIV-1 and noninfectious HBV DNA plasmid with EDTA and 0.05% sodium azide as a preservative.

MP LYS (Multiprep Lysis Reagent) 8 x 9.0 mL
Tris-HCl buffered solution with Dithiothreitol, Glycogen and Guanidine thiocyanate

X_n



Harmful

MP DIL	(Multiprep Specimen Diluent)	8 x 4.8 mL
Tris-HCl buffered solution with EDTA and 0.05% sodium azide as a preservative		
MP IC	(Multiprep Internal Control)	8 x 0.1 mL
Tris-HCl buffered solution with non-infectious internal control RNA transcripts for HCV and HIV-1 and DNA plasmid for HBV, Poly rA RNA, EDTA, indicator dye and 0.05% sodium azide as a preservative.		
MP (-) C	(Multiprep Negative (-) Control)	8 x 0.1 mL
Poly rA RNA, EDTA and 0.05% sodium azide as a preservative		
NHP	(Negative Plasma (Human))	16 x 1.6 mL
Human plasma, non-reactive by US FDA licensed tests for antibody to HIV-1/2, antibody to HCV, HIV-1 p24 antigen and HBsAg, with 0.1% ProClin® 300 as a preservative.		

COBAS AmpliScreen HIV-1 Test, version 1.5, 96 Tests

Amplification Reagents

HIV-1 MMX v1.5	(HIV-1 Master Mix, version 1.5)	8 x 0.7 mL
Bicine buffered solution with DMSO, glycerol, rTth DNA Polymerase, potassium acetate, primers, dNTPs, AmpErase and 0.05% sodium azide as a preservative		
HIV-1 Mn²⁺ v1.5	(HIV-1 Manganese Solution, version 1.5)	8 x 0.1 mL
Manganese solution with acetic acid, indicator dye and 0.05% sodium azide as a preservative		

COBAS AmpliScreen HIV-1 Detection Reagents, version 1.5

DN4 (Denaturation Solution) 1 x 100 Tests

EDTA

Thymol blue

Xi 1.6% (w/w) Sodium hydroxide



Harmful

IH PS1 v1.5 (HIV-1 Probe Suspension 1, version 1.5) 1 x 100 Tests

MES buffer solution containing capture oligonucleotides and magnetic microparticles with 0.05% sodium azide as a preservative

IH4 v1.5 (HIV-1 Probe Suspension 2, version 1.5) 1 x 100 Tests

Sodium phosphate buffer

34.7% Sodium thiocyanate

0.2% Solubilizer

Xn 34.7% (w/w) Sodium thiocyanate



Harmful

II PS1 (IC Probe Suspension 1) 1 x 100 Tests

MES buffer solution containing magnetic microparticles with capture oligonucleotides and 0.09% sodium azide as a preservative

II4 (IC Probe Suspension 2) 1 x 100 Tests

Sodium phosphate buffer containing 24.9% sodium thiocyanate



Harmful

CN4 (Avidin-Horseradish Peroxidase Conjugate) 2 x 100 Tests
Tris-HCl buffer solution containing Avidin-horseradish peroxidase conjugate,
bovine serum albumin, Emulsit 25 and phenol with ProClin 150 as a preservative

SB3 (Substrate A) 10 x 75 Tests
Citrate solution containing hydrogen peroxide with ProClin 150 as a preservative

SB (Substrate B) 10 x 75 Test

0.1% 3,3',5,5'-Tetramethylbenzidine (TMB)

40% Dimethylformamide (DMF)

T 40% (w/w) Dimethylformamide (DMF)



Toxic

R: 61-20/21-36 May cause harm to the unborn child. Harmful by inhalation
and in contact with skin. Irritating to eyes.

S: 53-45 Avoid exposure — obtain special instructions before use. In
case of accident or if you feel unwell, seek medical advice
immediately (show the label where possible).

COBAS AMPLICOR Wash Buffer, 500 Tests

WB (10X-Wash Concentrate) 2 x 250 Tests
Phosphate buffer solution containing detergent with ProClin 300 as a preservative

Storage Instructions

- A. Room Temperature is defined as 15 – 30°C.
- B. Do not freeze reagents.
- C. Store the following reagents at 2 – 8°C. Unopened, these reagents are stable until the expiration date indicated.

MP LYS, MP IC, MP (+) C, MP (-) C, MP DIL and NHP

HIV-1 MMX v1.5 and HIV-1 Mn²⁺ v1.5

IH PS1 v1.5, IH4 v1.5, II PS1 and II4

CN4, SB3 and SB

- D. Store **DN4** and **WB** at 2 – 25°C. **DN4** and **WB** are stable until the expiration dates indicated.
- E. Do not expose **SB3, SB** or Working Substrate to metals, oxidizing agents or direct sunlight.
- F. The following reagents are one time use. Discard any unused portion.

MP IC, MP (+) C, MP (-) C, MP DIL and NHP

HIV-1 Mn²⁺ v1.5, IH4 v1.5, II4 and SB

Precautions

For In Vitro Diagnostic use

- A. Specimens may be infectious. Use Universal Precautions when performing the assay.³⁰⁻³¹ Only personnel proficient in the use of the COBAS AmpliScreen System and trained in handling infectious materials should perform this procedure.
Thoroughly clean and disinfect all work surfaces with a freshly prepared solution of

0.5% sodium hypochlorite in distilled or deionized water. Follow by wiping down the surface with 70% ethanol.

- B. CAUTION: The Negative Human Plasma of this kit contains human blood products non-reactive by US FDA licensed tests for antibody to HIV-1/2, antibody to HCV, HIV-1 p24 antigen and HBsAg. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. All human blood-sourced materials should be considered potentially infectious and should be handled with Universal Precautions.** If spillage occurs, immediately disinfect, then wipe up with a 0.5% (final concentration) sodium hypochlorite solution (diluted bleach) or follow appropriate site procedures.
- C. Use routine laboratory precautions. Do not pipette by mouth. Do not eat, drink or smoke in designated work areas. Wear disposable gloves, laboratory coats and eye protection when handling specimens and kit reagents. Wash hands thoroughly after handling specimens and kit reagents.
- D. This product contains sodium azide as a preservative. Do not use metal tubing for reagent transfer. If solutions containing azide compounds are disposed of in a plumbing system, they should be diluted and flushed with generous amounts of running water. These precautions are recommended to avoid accumulation of deposits in metal piping in which explosive conditions could develop.
- E. Heparin has been shown to inhibit PCR. Do not use heparinized plasma with this procedure.**
- F. Use only supplied or specified required disposables to ensure optimal assay performance.
- G. Screw-cap tubes must be used for specimen and control preparation to prevent splashing and potential cross-contamination of specimens and controls.
Do not use snap cap tubes.
- H. Adequately vortex, where specified, to ensure optimal assay performance.
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- I. Handle all materials containing specimens or controls according to Good Laboratory Practices in order to prevent cross-contamination of specimens or controls.
 - J. Before use, visually inspect each reagent bottle to ensure that there are no signs of leakage and/or abnormal color. If there is any evidence of leakage and/or abnormal color, do not use that bottle for testing.
 - K. Dispose of all materials that have come in contact with specimens and reagents in accordance with country, federal, state and local regulations.
 - L. Do not use a kit after its expiration date. DO NOT interchange, mix, or combine reagents from kits with different master lot numbers. Do not use expired reagents.
 - M. Material Safety Data Sheets (MSDS) are available on request.
 - N. Supplies and equipment must be dedicated to each pre-amplification activity should not be used for other activities or moved between areas. ***Fresh, clean gloves must be worn in each area and must be changed before leaving that area.*** Equipment and supplies used for reagent preparation must not be used for specimen preparation activities or for pipetting or processing amplified DNA or other sources of target DNA. Post-amplification supplies and equipment must remain in the Post-Amplification Area at all times.
 - O. Avoid contact of **MP LYS, HIV-1 MMX v1.5, HIV-1 Mn²⁺ v1.5, IH4 v1.5, II4, DN4, CN4, SB3, SB** and Working Substrate (mixed **SB3** and **SB** reagent) with the skin, eyes or mucous membranes. ***If contact does occur, immediately wash with large amounts of water, otherwise, burns can occur.*** If these reagents are spilled, dilute with water before wiping dry. ***Do not allow MP LYS, which contains guanidine thiocyanate, or IH4 v1.5 and II4, which contain sodium thiocyanate, to contact sodium hypochlorite (bleach) solution. This mixture can produce a highly toxic gas.***
 - P. **SB and Working Substrate contain dimethylformamide, which has been reported to be toxic in high oral doses, and may be harmful to the unborn child. Skin contact, inhalation of fumes and ingestion should be avoided. If skin**

contact occurs, wash thoroughly with soap and water and seek medical advice immediately.

- Q. Refer to “Precautions” in other COBAS AmpliScreen package inserts, COBAS Pooling System Guide, and the *Operator’s Manuals* for the AMPLILINK and COBAS AMPLICOR Analyzer.
- R. Closely follow procedures and guidelines provided to ensure that the specimen and control preparation is performed correctly. Any deviation from the given procedures and guidelines may affect optimal assay performance.

Reagent Preparation

MP IC, MP (+) C, MP (-) C, MP DIL and NHP

- Warm **MP IC, MP(+)** C, **MP(-)** C, **MP DIL** and **NHP** to room temperature before use by using a 37°C incubator on laboratory bench top.

Working Lysis Reagent

- Warm **MP LYS** to 25 – 37°C to dissolve precipitate (maximum 30 minutes). Mix thoroughly until the crystals are dissolved. Before use, visually verify that crystals are dissolved and examine each bottle of **MP LYS** against a white background. Discard **MP LYS** if there is a yellow color or signs of leakage.
- Vortex **MP IC** briefly before use. Tap vial to collect the solution in the base. Pipette 100 µL **MP IC** into 1 bottle **MP LYS**). Cap the **MP LYS** bottle and vortex briefly. The pink color confirms that the **MP IC** has been added to the **MP LYS**. Discard the remaining **MP IC**.
- Store Working Lysis Reagent at room temperature. Use within 4 hours of preparation.

Working Amplification Master Mix

- Prepare Working Master Mix in a template-free area (e.g., in a dead air box).
Reagent preparation area must be clean and disinfected in accordance with methods outlined in Precautions (Item A). Failure to do so may result in reagent contamination.
- Pipette 100 µL **HIV-1 Mn²⁺ v1.5** into 1 bottle **HIV-1 MMX v1.5** Recap **HIV-1 MMX v1.5** bottle and mix well by inverting 10 – 15 times. The pink color confirms that the **HIV-1 Mn²⁺ v1.5** has been added to the **HIV-1 MMX v1.5**. Discard the remaining **HIV-1 Mn²⁺ v1.5**. *Do not vortex the Working Master Mix.* These reagents do not need to be at room temperature before use.
- Store at 2 – 8°C and use within 4 hours of preparation.

Working Probe Suspension Detection Reagents

- Prepare Working HIV-1 Probe Suspension: Mix **IH PS1, v1.5** well by vortexing briefly to suspend the microparticles. Pipette 2.5 mL **IH PS1, v1.5** into one **IH4, v1.5** cassette.
- Prepare Working IC Probe Suspension: Mix **II PS1** well by vortexing briefly to suspend the microparticles. Pipette 2.5 mL **II PS1** into one **II4** cassette.
- Both Working Probe Suspension Detection Reagents are stable for 30 days at 2 – 8°C. Working Reagents can be used for a maximum of six instrument cycles (12 hours per cycle). Mixing occurs automatically on the COBAS AMPLICOR Analyzer.
- Store Working Probe Suspension Detection Reagents at 2 – 8°C between instrument cycles. Remove from refrigerator 30 minutes before use on the COBAS AMPLICOR Analyzer.

DN4 — Denaturation Reagent and CN4 Conjugate Reagent

- Once opened, **DN4** and **CN4** are stable for 30 days at 2 – 8°C, or until the expiration date, whichever comes first. Both DN4 and CN4 can be used for a maximum of six instrument cycles (12 hours per cycle).
- Store **DN4** and **CN4** at 2 – 8°C between instrument cycles. Remove from refrigerator 30 minutes before use on the COBAS AMPLICOR Analyzer.

Working Substrate Reagent

- Working Substrate must be prepared each day by pipetting 5 mL **SB** into one **SB3** cassette. Pipette up and down at least 5 times to mix.
- Working Substrate is stable on the COBAS AMPLICOR Analyzer for a maximum of 16 hours.
- Do not expose **SB3**, **SB**, or Working Substrate to metals, oxidizing agents or direct light.

Wash Buffer Reagent

- Examine **WB** before dilution and if necessary, warm at 30 – 37°C to dissolve any precipitate. Add 1 volume of **WB** to 9 volumes of distilled or deionized water. Mix well. Keep a minimum of 3 – 4 liters of Working Wash Buffer (1X) in the Wash Buffer Reservoir of the COBAS AMPLICOR Analyzer at all times.
- Working Wash Buffer (1X) should be stored at 2 – 25°C in the COBAS AMPLICOR Wash Buffer Reservoir and is stable for 2 weeks from the date of preparation.

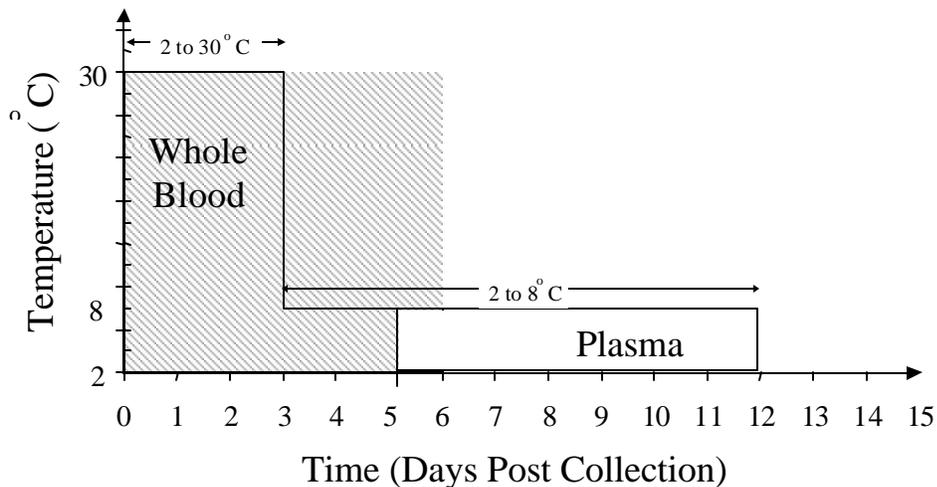
70% Ethanol

- Prepare 70% ethanol fresh daily.
- One mL 70% ethanol is needed for each specimen and control processed. For example, mix 11.7 mL 90% ethanol and 3.3 mL of distilled or deionized water for every 12 specimens and controls to be processed.

Specimen Collection, Storage and Pooling

Note: Handle all specimens as if they are potentially infectious agents.

- A. EDTA, CPD, CPDA-1, CP2D, ACD-A and 4% Sodium Citrate may be used with the COBAS AmpliScreen HIV-1 Test, v1.5. Follow sample tube manufacturer's instructions.
- B. Blood collected in EDTA may be stored for up to 72 hours from time of draw at 2 – 30°C, followed by an additional two days at 2-8°C. For storage longer than five days, remove the plasma from the red blood cells by centrifugation at 800 – 1600 $\times g$ for 20 minutes. Following removal, plasma may be stored at 2 – 8°C for an additional seven days. Alternatively, plasma may be stored at = -18°C for up to one month.



- C. Blood collected in CPD, CPDA-1, or CP2D may be stored for up to 72 hours at 1 – 24°C. Following centrifugation of the CPD, CPDA-1, or CP2D samples at 800 – 1600 $x g$ for 20 minutes, plasma may be stored at 1 – 6°C for an additional 7 days from the date the plasma was removed from the red blood cells. Plasma separated from the cells may be stored at = -18°C for up to one month.
- D. ACD-A or 4% sodium citrate anticoagulated apheresis plasma can be stored at 1 – 6°C for up to 6 hours, followed by subsequent storage at = -18°C for up to one month.
- E. Do not freeze whole blood.
- F. **Heparin has been shown to inhibit PCR. Use of heparinized specimens is not recommended.**
- G. Warm pooled or individual donor specimens to room temperature before using.
- H. Covered Archive Plates may be stored at 2 – 8°C for up to 7 days from the date the plasma was removed from the red blood cells.
- I. No adverse effect on assay performance was observed when plasma specimens were subjected to three freeze-thaw cycles.
- J. Thaw frozen specimens at room temperature before using.
- K. The user should validate other collection and storage conditions. If specimens are to be shipped, they should be packaged and labeled in compliance with applicable federal and international regulations covering the transport of clinical specimens and etiologic agents.³²
- L. **False positive results may occur if cross contamination of specimens is not adequately controlled during specimen handling and processing.**
- M. Specimen Pooling

The COBAS AmpliScreen Pooling System performs barcode scanning and pooling operations that combine aliquots from 24 individual samples into a single Primary

Pool that is used for testing. The pooling algorithm requires preparation of Secondary Pools as well as individual specimens for follow-up testing in the event a Primary Pool tests positive. If less than 24 specimens are available, testing is performed using the individual specimens.

The user must validate other pooling algorithms and equipment.

Procedural Notes

A. Run Size

1. Each kit contains reagents sufficient for eight 12-specimen runs, which may be performed separately or simultaneously. At least one preparation of the COBAS AmpliScreen Multiprep Negative (–) Control and one preparation of the COBAS AmpliScreen Multiprep Positive (+) Control must be included in each run (see “*Quality Control*” section).
2. The Specimen Preparation and Amplification Reagents are packaged in eight single-use bottles. The Multiprep Negative (–) and Multiprep Positive (+) Controls are packaged in single-use vials. For the most efficient use of reagents, specimens and controls should be processed in batches that are multiples of 12.
3. The use of sterile gauze, when uncapping sample tubes may reduce the potential for cross contamination between specimens.

B. Equipment

1. Prepare the COBAS AMPLICOR Analyzer and AMPLILINK Data Station for use according to instructions in the *Operator’s Manuals* for the AMPLILINK software and the COBAS AMPLICOR Analyzer.
2. Prepare the Hamilton Microlab AT plus 2 System and SUNPLUS Data Station for use according to instructions in the *Operator’s Manuals*.

3. Pre-cool the high-speed centrifuge and rotor to 2 – 8°C. See operating instructions for the high-speed centrifuge for details.
4. Perform manufacturer recommended maintenance and calibration on all instruments, including pipettors, to ensure proper functioning.

C. Reagents

1. All reagents, *except HIV-1 MMX, v1.5* and *HIV-1 Mn²⁺, v1.5*, must be at room temperature before use. Visually examine reagents for sufficient volume before beginning the test procedure. See “Reagent Preparation” for specific reagent storage conditions.
2. Add all reagents using a pipettor capable of delivering specified volume with ± 3% accuracy and a precision of ≤ 5% CV. Check pipettor functionality and calibrate as recommended by pipettor manufacturer.
3. Prepare Working Master Mix in a template-free area (e.g., in a dead air box).
Reagent preparation area must be clean and disinfected in accordance with methods outlined in Precautions (Item A). Failure to do so may result in reagent contamination.
4. Prepare 70% ethanol fresh each day.
5. Check expiration date of opened or Working Reagents before loading on the COBAS AMPLICOR Analyzer.
6. Check to ensure that all reagents used are of the same master lot of kit reagents.

D. Workflow

1. To minimize the possibility of laboratory areas becoming contaminated with amplicon, the laboratory area should be separated into several distinct areas organized around Pre-Amplification and Post-Amplification. Personnel should use proper anti-contamination safeguards when moving between areas.

2. The Pre-Amplification Area should have a template-free area for preparation of Working Master Mix and an amplicon free area for specimen and control preparation.
3. The Post Amplification Area should have a COBAS AMPLICOR Analyzer(s) and AMPLILINK Data Station(s) with additional area for preparing Working Amplification and Detection Reagents.
4. Pipettors and other supplies should be dedicated to a specific area. Samples, equipment and reagents should not be returned to the area where a previous step was performed.

E. Temperature

Room temperature is defined as 15° to 30°C.

F. Vortexing

Proper vortexing during sample preparation is important to ensure homogeneous mixture after additions of reagents.

G. Pipetting

1. Pooled or individual plasma specimens must be at room temperature before pipetting.
2. Use a clean pipette tip or disposable transfer pipette with each specimen or control. Use aerosol barrier or positive displacement RNase-free tips.
3. Confirm that all pipettors are correctly set to dispense the specified volumes in accordance with the specimen preparation procedures and guidelines.

H. Specimen Processing

1. Screw-cap tubes must be used for specimen and control preparation to prevent splashing and potential cross-contamination of specimens and controls. Do not use snap cap tubes.
2. Avoid contaminating gloves when manipulating specimens.
3. Specimens and controls should be prepared in a laminar flow hood. Failure to do so may result in sample contamination. Specimen and control preparation area must be cleaned and disinfected in accordance with methods outlined in “Precautions” (Item A).

I. Decontamination

Thoroughly clean and disinfect all work surfaces with a freshly prepared solution of 5% sodium hypochlorite in distilled or deionized water. Follow by wiping down the surface with 70% ethanol.

Instructions for Use

The Multiprep Specimen Processing Procedure is used for extracting nucleic acid from pooled specimens. The Standard Specimen Processing Procedure is used for extracting nucleic acid from individual specimens as well as for tertiary resolution.

The Multiprep and the Standard Specimen Processing Procedures are generic nucleic acid extraction procedures and can be used for the extraction HIV-1 RNA, HCV RNA, and/or HBV DNA. A single extraction is sufficient for multiple assays.

Workflow can be performed on the same day or over multiple days under the following conditions:

Amplification, Hybridization and Detection of Stored Processed Specimens

Amplification, hybridization and detection can occur on the same day as specimen processing or on a subsequent day. If amplification, hybridization and detection are to be done on a subsequent day, perform the Multiprep Specimen Processing Procedure described in steps B1 through B21 or the Standard Specimen Processing Procedure described in steps B22 through B38. Store the processed specimens and controls as indicated. On the subsequent day, begin with Step A (Reagent Preparation — Working Master Mix), thaw processed specimens and controls at room temperature, and continue with Step B39.

Hybridization and Detection of Stored Denatured Amplicon

Hybridization and detection of the denatured amplicon may occur on the same day as amplification or on a subsequent day. If hybridization and detection are to be done on a subsequent day, the denatured amplicon may be left on-board the COBAS AMPLICOR Analyzer for not more than 24 hours before starting the hybridization and detection steps.

Alternatively, the denatured amplicon may be stored at 2 – 8°C for not more than five days before starting the hybridization and detection steps.

A. Reagent Preparation — Working Master Mix

Performed in Pre-Amplification — Reagent Preparation Area (e.g., dead air box)

- A1. Determine the appropriate number of A-ring(s) needed for specimen and control testing.
- A2. Place the A-ring(s) on the A-ring holder(s).
- A3. For each A-ring, prepare one bottle of Working Master Mix.
- A4. Pipette 50 µL Working Master Mix into each A-tube. Discard unused Working Master Mix. Do not close the covers of the A-tubes at this time.
- A5. Place the A-ring containing Working Master Mix in a sealable plastic bag and seal the plastic bag. Record the assay name (HIV-1) and the time the Working Master Mix was prepared.
- A6. Store the A-ring(s) containing Working Master Mix at 2 – 8°C until specimen and control preparation is completed. The A-rings with Working Master Mix must be used within 4 hours of preparation.
- A7. Decontaminate area. See “*Procedural Notes.*”

B. Specimen and Control Preparation

Performed in Pre-Amplification — Specimen and Control Preparation Area

Multiprep Specimen Processing Procedure

- B1. Pipette 1000 µL of each pool into an appropriately labeled screw-capped tube using the COBAS AmpliScreen Pooling System, a hand-held pipettor or other user-validated method. Cap the tubes.

- B2. Vortex NHP briefly.
- B3. For each Negative and Positive Control pipette 1000 µL NHP into an appropriately labeled screw-capped tube. Cap the tubes.
- B4. Use a permanent marker to make an orientation mark on each tube.
- B5. Place the specimen and control tubes into the pre-cooled high-speed centrifuge with the orientation marks facing outward, so that the orientation marks will align with the pellets formed during centrifugation.
- B6. Centrifuge specimens and control tubes at 23,000 – 24,000 x g for 60 ± 4 minutes at 2 – 8°C. The pellet will form on the outer wall as indicated by the orientation mark. **Note: The 60 ± 4 minutes begins when the centrifuge reaches 23,000 – 24,000 x g.**
- B7. Remove the tubes from the centrifuge and remove the caps. Slowly aspirate 900 µL of the supernatant from each centrifuged tube leaving approximately 100 µL of supernatant. Avoid contact with the pellet. Discard the supernatant and pipette tip appropriately. Use a fresh pipette tip for each tube.
- B8. Prepare a Working Lysis Reagent bottle for every batch of 12 specimens and controls to be processed.
- B9. Pipette 600 µL Working Lysis Reagent into each specimen and control tube. Cap and vortex tubes briefly.

B10. Prepare Controls as follows:

Negative Control

Vortex **MP (-) C** briefly. Tap vial to collect the solution in the base. Pipette 20 μ L **MP (-) C** to the tube labeled “MP (-) C” containing Working Lysis Reagent and **NHP**. Cap the tube and vortex briefly.

Positive Control

Vortex **MP (+) C** briefly. Tap vial to collect the solution in the base. Pipette 20 μ L **MP (+) C** to the tube labeled “MP (+) C” containing Working Lysis Reagent and **NHP**. Cap the tube and vortex for briefly.

- B11. Incubate all tubes for 10 to 15 minutes at room temperature after adding Working Lysis Reagent to the last tube. After the incubation period, briefly vortex all tubes.
- B12. Pipette 700 μ L of isopropanol into each tube. Cap the tubes and vortex briefly.
- B13. Place the tubes into a microcentrifuge with the orientation marks facing outward to align with the pellets that will form. Centrifuge at $14,250 \pm 1750 \times g$ for 15 – 20 minutes at room temperature.
- B14. Slowly aspirate the supernatant from each tube. Remove as much liquid as possible without disturbing the pellet.
- B15. Pipette 1.0 mL of 70% ethanol into each tube. Cap the tubes and vortex briefly.
- B16. Place the tubes into a microcentrifuge with the orientation marks facing outward to align with the pellets that will form. Centrifuge at $14,250 \pm 1750 \times g$ for 5 – 10 minutes at room temperature.
- B17. Slowly aspirate the supernatant from each tube using a fine-tip disposable transfer pipette. Remove as much liquid as possible without disturbing the pellet. Use a new transfer pipette for each tube.

- B18. Using a new transfer pipette for each tube, repeat Step B17 to remove as much of the remaining supernatant as possible without disturbing the pellet. ***Residual ethanol can inhibit amplification.***
- B19. Pipette 200 µL **MP DIL** into each tube. Use a pipette tip to break apart the pellet. This can be done by aspirating 30 – 40 µL of the diluent in the tip and scraping the sides and base of the tube in an up/down motion for at least 10 seconds. Cap the tubes and vortex briefly to resuspend the extracted RNA. Note that some insoluble material may remain.
- B20. At this point amplification of the processed specimens and controls must be started within 2 hours. If not, the processed specimens and controls can be stored at -70°C or colder for up to one month. ***Thawing should be completed within one hour at room temperature.***
- B21. Proceed to step **B39, “Loading the A-ring”**

Standard Specimen Processing Procedure

- B22. Pipette 200 µL of each specimen into an appropriately labeled screw-capped tube using the COBAS AmpliScreen Pooling System, a hand-held pipettor or other user-validated method. Cap the tubes.
- B23. Vortex NHP briefly.
- B24. For each Negative and Positive Control pipette 200 µL NHP into appropriately labeled screw-capped tubes. Cap the tubes.
- B25. Use a permanent marker to make an orientation mark on each tube.
- B26. Prepare a Working Lysis Reagent bottle for every 12 specimens and controls to be processed.
- B27. Pipette 600 µL Working Lysis Reagent into each tube. Cap and vortex tubes briefly.

B28. Prepare Controls as follows:

Negative Control

Vortex **MP (-) C** briefly. Tap vial to collect the solution in the base. Pipette 20 µL **MP (-) C** into the tube labeled “MP (-) C” containing Working Lysis Reagent and **NHP**. Cap the tube and vortex briefly.

Positive Control

Vortex **MP (+) C** briefly. Tap vial to collect the solution in the base. Pipette 20 µL **MP (+) C** into the tube labeled “MP (+) C” containing Working Lysis Reagent and **NHP**. Cap the tube and vortex briefly.

- B29. Incubate all tubes for 10 – 15 minutes at room temperature after adding Working Lysis Reagent to the last tube. After the incubation period, briefly vortex all tubes.
- B30. Pipette 800 µL of isopropanol into each tube. Cap the tubes and vortex briefly.
- B31. Place the tubes into a microcentrifuge with the orientation marks facing outward to align with the pellets that will form. Centrifuge at $14,250 \pm 1750 \times g$ for 15 – 20 minutes at room temperature.
- B32. Slowly aspirate the supernatant from each tube. Remove as much liquid as possible without disturbing the pellet.
- B33. Pipette 1.0 mL of 70% ethanol into each tube. Cap the tubes and vortex briefly.
- B34. Place the tubes into a microcentrifuge with the orientation marks facing outward to align with the pellets that will form. Centrifuge at $14,250 \pm 1750 \times g$ for 5 – 10 minutes at room temperature.
- B35. Slowly aspirate the supernatant from each tube using a fine-tip disposable transfer pipette. Remove as much liquid as possible without disturbing the pellet. Use a new transfer pipette for each tube.

- B36. Using a new transfer pipette for each tube, repeat Step B35 to remove as much of the remaining supernatant as possible without disturbing the pellet. ***Residual ethanol can inhibit amplification.***
- B37. Pipette 200 µL MP DIL into each tube. Use a pipette tip to break apart the pellet. This can be done by aspirating 30-40 µL of the diluent in the tip and scraping the sides and base of the tube in an up/down motion for at least 10 seconds. Cap the tubes and vortex briefly to resuspend the extracted RNA. Note that some insoluble material may remain.
- B38. At this point amplification of the processed specimens and controls must be started within 2 hours. If not, the processed specimens and controls can be stored at -70°C or colder for up to one month. ***Thawing should be completed within one hour at room temperature.***

Loading the A-ring

- B39. Create an A-ring worklist record for each A-ring to identify the A-tube with the appropriate control or specimen to be pipetted.
- B40. If processed specimens and controls were stored frozen, thaw at room temperature before proceeding. Briefly vortex the processed specimens and controls.
- B41. Pipette 50 µL of each processed specimen and control into the appropriate A-tube containing HIV-1 Working Master Mix. Use the A-ring worklist record to ensure the appropriate specimen or control is added to the correct A-tube position for each A-ring.
- B42. Cap the A-tubes.
- B43. Transfer the A-ring with sealed tubes containing the processed specimens and controls in Working Master Mix to the Amplification/Detection Area. Proceed to Part C.

Note: Amplification must begin within 45 minutes from when the first specimen or control in the A-ring is added to the Working Master Mix.

C. Reverse Transcription, Amplification and Detection

Performed in Post-Amplification — Amplification/Detection Area

- C1. Perform Daily Instrument Maintenance as outlined in the *Operator's Manual* for the COBAS AMPLICOR Analyzer including:
 - a. Wipe D-cup handler tip with a lint-free moist cloth and dry.
 - b. Wipe initialization post with a lint-free moist cloth and dry.
- C2. Prior to each run
 - a. Check waste container and empty if necessary.
 - b. Check Wash Buffer Reservoir and add prepared Wash Buffer if necessary.
 - c. Replace used D-cup racks.
 - d. Prime the COBAS AMPLICOR Analyzer.
- C3. Instrument Loading and System Operation
 - a. Prepare enough of the following detection reagent cassettes to complete the workload: Working HIV-1 Probe Suspension Reagent (**IH4, v1.5**), Working IC Probe Suspension Reagent (**II PS1**), Working Substrate (**SB3**), Denaturation Reagent (**DN4**), and Conjugate Reagent (**CN4**).
 - b. Place the **IH4, v1.5** and **II PS1** cassettes in the test-specific reagent rack.
 - c. Place **DN4, CN4** and **SB3** cassettes in the generic reagent rack. Record on the cassette the date when each cassette was opened.
 - d. Identify the reagent racks as generic or test specific using the COBAS AMPLICOR Analyzer barcode scanner for the AMPLILINK software, as described in the *Operator's Manual* for AMPLILINK software.

- e. Configure the reagent racks by entering the reagent positions and lots using the COBAS AMPLICOR Analyzer barcode scanner for the AMPLILINK software, as described in the *Operator's Manual* for AMPLILINK software.
- f. Load the reagent racks onto the analyzer using the COBAS AMPLICOR Analyzer barcode scanner for the AMPLILINK software, as described in the *Operator's Manual* for AMPLILINK software. Make sure that each reagent cassette is in its assigned position and that each cassette fits tightly into its rack.
- g. Place the D-cup rack on the D-cup platform. Two D-cups are required for each A-tube and two D-cups are required for each Working Substrate cassette to allow for blanking by the COBAS AMPLICOR Analyzer, as described in the *Operator's Manual* for the COBAS AMPLICOR Analyzer.
- h. Place the A-ring into the thermal cycler segment of the COBAS AMPLICOR Analyzer and close the cover on the thermal cycler segment.
- i. Load the A-ring into the COBAS AMPLICOR Analyzer using the Analyzer barcode scanner for the AMPLILINK software, as described in the *Operator's Manual* for AMPLILINK software.
- j. Create an A-ring order, using the AMPLILINK software, as described in the *Operator's Manual* for AMPLILINK software. Use the A-ring worklist record created for specimen processing to assist in entering the A-ring order.
- k. Repeat steps h. through j. above to load a second A-ring on the COBAS AMPLICOR Analyzer.
- l. Start the COBAS AMPLICOR Analyzer as described in the *Operator's Manual* for AMPLILINK software.
- m. Wait for the COBAS AMPLICOR Analyzer to indicate that the load check has passed.

Note: The required quantity of each detection reagent is automatically calculated by the COBAS AMPLICOR Analyzer during the Load Check to determine if sufficient reagents are available for the requested tests.

- n. The COBAS AMPLICOR Analyzer automatically performs reverse transcription, amplification and detection. Results are expressed as absorbance values at 660 nm and as positive or negative.
- o. For each run, print the AMPLILINK A-ring Results Report and the Run Log and retain these along with the A-ring worklist. Compare the A-ring worklist record with the AMPLILINK A-ring Results Report and verify that the A-ring ID, instrument serial number, and specimen IDs are identical. Reconcile the Run Log with the A-ring worklist to account for all A-ring IDs associated with each run. If there are discrepancies, perform follow-up investigation.

Quality Control Procedures

1. At least one Multiprep (-) Control and one Multiprep (+) Control must be processed with each on an A-ring.

- a. Negative Control

The absorbance for the **MP (-) C** should be less than 0.2 at 660 nm and its associated **MP IC** should be greater than or equal to 0.2 for the Negative Control to be valid. If the absorbance value for the **MP (-) C** is greater than or equal to 0.2 and/or its associated **MP IC** is less than 0.2, the entire run is invalid, and the entire test procedure (sample and control preparation, amplification and detection) must be repeated.

- b. Positive Control

The absorbance for the **MP (+) C** should be greater than or equal to 1.0 at 660 nm and its associated **MP IC** should be greater than or equal to 0.2 at 660 nm for the Positive Control to be valid. If the absorbance value for the **MP (+) C** is less than

1.0 and/or its associated **MP IC** is less than 0.2, the entire run is invalid, and the entire test procedure (specimen and control preparation, amplification and detection) must be repeated.

Summary of Control Acceptance Criteria

	HIV-1 Result		IC Result	
	A ₆₆₀	Comment	A ₆₆₀	Comment
Negative Control	<0.2	Negative	≥0.2	Valid
Positive Control	≥1.0	Positive	≥0.2	Valid

2. Flags and comments may be generated by the COBAS AMPLICOR Analyzer during a run. The Operator must check the run printout(s) for flags and comments to verify that the run is valid. Refer to the *Operator's Manuals* for the AMPLILINK software and the COBAS AMPLICOR Analyzer for interpretation of flags and comments.

3. External Control

If an External Control (i.e., an additional run control other than the Multiprep (+) Control or Multiprep (-) Control) is required by the laboratory, the External Control should meet regulatory requirements for such controls. The absorbance of the HIV-1 External Control should be equal to or greater than 0.2 at 660 nm, irrespective of the MP IC absorbance. If the absorbance of the HIV-1 External Control does not meet the above criterion, the negative results for specimens in the associated run may be invalidated. However, positive results for specimens in such a run should not be invalidated solely on the basis of the results obtained for an External Control; those positive results should remain the test of record. The laboratory should follow its established Standard Operating Procedure for the appropriate action.

Interpretation of Results

1. Flags and comments may be generated by the COBAS AMPLICOR Analyzer during a run. The Operator must check the run printout(s) for flags and comments to verify that the run is valid. Refer to the *Operator's Manuals* for the AMPLILINK software and the COBAS AMPLICOR Analyzer for interpretation of flags and comments.

2. Specimen Results

Two absorbance values are obtained for each specimen: one for the HIV-1 target and one for the internal control (**MP IC**). For a sample with an absorbance less than 0.2, the **MP IC** absorbance for that specimen must be greater than or equal to 0.2 at 660 nm for a valid negative specimen test result. If the absorbance for the HIV-1 target is greater than or equal to 0.2, the **MP IC** result is disregarded and the test result is valid and positive.

3. For a valid run, results are interpreted as follows:

HIV-1 Result		IC Result		Interpretation
A ₆₆₀	Comment	A ₆₆₀	Comment	
< 0.2	NEGATIVE	≥ 0.2	VALID	Specimen is negative for HIV-1 RNA.
< 0.2	NEGATIVE	< 0.2	INVALID	Invalid result. Repeat entire test procedure for invalid specimen.
≥ 0.2	POSITIVE	ANY	VALID	Specimen is positive for HIV-1 RNA

Invalid Test Runs

When invalid Positive or Negative Control results are obtained on an A-ring, that run is invalid. Repeat the entire test procedure for the associated specimens (including specimen and control preparation, amplification and detection) in the run by processing another aliquot of the original plasma specimens.

With the exception of instrument failures subsequent to denaturation of amplicon, an instrument failure during a test run, as indicated by system error messages, also constitutes an invalid test run. In such instances, repeat the test procedure for the associated controls and specimens (amplification and detection) in the run by processing another aliquot of the processed specimen.

For instrument failures subsequent to successful denaturation of amplicon, it is not necessary to repeat the entire test procedure for the associated specimens. In such instances, the denatured amplicon may be redetected by the COBAS AMPLICOR Analyzer. The denatured amplicon may be left on-board the COBAS AMPLICOR Analyzer for not more than 24 hours before continuing with the hybridization and detection steps. Alternatively, the denatured amplicon may be stored at 2 – 8°C for not more than five days before continuing with the hybridization and detection steps.

Invalid Specimen Results

For specimen(s) that are invalid, perform repeat testing in single on the remaining replicate tube(s). The test result for the pool or individual donor specimen is based only on the repeat valid test result. If the last available replicate of a pooled specimen gives an invalid result, each individual donor specimen in that pool should be tested. If an individual donor specimen gives an invalid result, the test result for that individual donor specimen should be considered invalid for HIV-1 RNA.

Results of Pooled Donor Specimens

The testing algorithm for testing of pooled samples for the COBAS AmpliScreen HIV-1 Test, v1.5 requires a single level of testing for Primary Pools that are negative for HIV-1 RNA and three levels of testing (Primary Pool, Secondary Pool and tertiary resolution) for Primary Pools that are positive for HIV-1 RNA.

Negative Primary Pools

When the Primary Pool is negative, report the results for all associated individual donor specimens in that Primary Pool as “HIV-1 RNA Negative.”

Positive Primary Pools — Secondary Pool Testing

When the Primary Pool is positive, prepare four Secondary Pools containing the associated donor specimens. The Secondary Pools must be processed using the Multiprep Specimen Processing Procedure.

- If one or more of the Secondary Pools tests positive, report the results for the donor specimens in the negative Secondary Pools as “HIV-1 RNA Negative.” For positive Secondary Pools, proceed to the section entitled “***Positive Primary Pool, Positive Secondary Pools - Tertiary Resolution Testing.***”
- If all four Secondary Pools are negative, the individual donor specimens in that Primary Pool may be reported as “HIV-1 RNA Negative.”
- As part of an overall Quality Assurance program, you may wish to conduct additional testing to determine the cause of the initial positivity of the Primary Pool.

Positive Primary Pool, Positive Secondary Pools — Tertiary Resolution Testing

For a positive Secondary Pool, test each of the individual donor specimens in that Secondary Pool. The individual donor specimens must be processed using the Standard Specimen Processing procedure.

- If one or more of the individual donor specimens is positive, the positive donor specimen(s) is (are) reported as “HIV-1 RNA Positive” and the remaining negative donor specimens associated with the positive Secondary Pool are reported as “HIV-1 RNA Negative.”
- If all of the individual donor specimens in that Secondary Pool test negative, the donor specimens in the Secondary Pool may be reported as “HIV-1 RNA Negative.”
- As part of an overall Quality Assurance program, you may wish to conduct additional testing to determine the cause of the positivity of the Primary Pool and Secondary Pools.

Results of Individual Donor Samples

If an individual donor specimen is positive, the positive donor specimen is reported as “HIV-1 RNA Positive.”

If an individual donor specimen is negative, the negative donor specimen is reported as “HIV-1 RNA Negative.”

Procedural Limitations

1. This test has been evaluated only for use in combination with the COBAS AmpliScreen Multiprep Specimen Preparation and Control Kit, COBAS AMPLICOR Analyzer, the Hamilton Microlab AT plus 2 Pipettor for the automated preparation of plasma pools.
2. Eight Group O culture specimens were only evaluated as diluted samples due to limited specimen volume. All HIV-1 Group O specimens tested were found to be HIV-1 p24 antigen positive, however, only five (63%) were detected by the COBAS AmpliScreen HIV-1 Test, v1.5. This data indicates that the COBAS AmpliScreen HIV-1 Test, v1.5 will not consistently detect HIV-1 RNA in all Group O specimens.
3. This COBAS AmpliScreen HIV-1 Test, v1.5 is intended to be used in conjunction with licensed tests for detecting antibodies to HIV-1. The COBAS AmpliScreen HIV-1 Test, v1.5 may not be used to replace HIV-1 antibody detection tests such as EIA or Western Blot (See Performance Characteristics section, Tables 12 and 13).
4. ***Heparin inhibits PCR; specimens collected using heparin as the anticoagulant should not be used with the COBAS AmpliScreen HIV-1 Test, v1.5.***
5. Reliable results are dependent on adequate specimen collection and proper transport procedures.
6. Detection of HIV-1 RNA is dependent on the number of virus particles present in the specimen and may be affected by specimen collection methods, patient factors (i.e., age, presence of symptoms), and/or stage of infection and pool size.

7. Only the Hamilton Microlab AT plus 2 Pipettor has been validated for use with the COBAS AmpliScreen HIV-1 Test, v1.5 for the automated preparation of plasma pools. Adhere to the hardware instructions and safety precautions outlined in the User Manual for the Hamilton Microlab AT plus 2 Pipettor.

Performance Characteristics

Reproducibility

The reproducibility of the COBAS AmpliScreen HIV-1 Test, v1.5 was established by testing two six-member EDTA plasma panels with known concentrations of HIV-1. Panel One was tested using the Multiprep Specimen Processing Procedure. Panel One was comprised of HIV-1 RNA positive samples at concentrations of 10, 25, 50, 75, and 25,000 copies/mL and one HIV-1-negative sample. Panel Two was tested using the Standard Specimen Processing Procedure. Panel Two was comprised of HIV-1 positive samples at concentrations of 50, 100, 150, 250, and 25,000 copies/mL and one HIV-1 negative sample.

Testing was performed at three sites with two operators at each site using five COBAS AmpliScreen HIV-1 Test, v1.5 kit lots. Each operator used a dedicated COBAS AMPLICOR Analyzer throughout the study. Each operator was provided panel sets that had been randomized and labeled in blinded fashion.

All valid reproducibility data were evaluated by calculating the percentage of correct results for each panel member. The data were analyzed by site, lot, testing day, run, and operator for each Specimen Processing Procedure (Multiprep and Standard).

The reproducibility study for the COBAS AmpliScreen HIV-1 Test, version 1.5 demonstrated consistency by lot and site for both the Multiprep and Standard Specimen Processing Procedures as seen in Table 1 and 2 below.

Table 1: Reproducibility Results — Multiprep Specimen Processing Procedure

Results By Lot (# Positive / # Tested)						
	Negative	10 c/mL	25 c/mL	50 c/mL	75 c/mL	25,000 c/mL
Lot #1 (%)	1/88 (1%)	51/90 (57%)	77/90 (86%)	86/90 (96%)	89/89 (100%)	90/90 (100%)
Lot #2 (%)	0/89 (0%)	47/90 (52%)	72/90 (80%)	83/90 (92%)	88/90 (98%)	90/90 (100%)
Lot #3 (%)	2/90 (2%)	50/89 (56%)	80/89 (90%)	88/89 (99%)	88/90 (98%)	90/90 (100%)
Lot #4 (%)	0/90 (0%)	45/90 (50%)	78/90 (87%)	84/90 (93%)	90/90 (100%)	90/90 (100%)
Lot #5 (%)	0/89 (0%)	51/89 (57%)	73/89 (82%)	83/90 (92%)	90/90 (100%)	90/90 (100%)
Results By Site (# Positive / # Tested)						
Site #1 (%)	3/150 (2%)	72/150 (48%)	133/150 (89%)	142/150 (95%)	149/150 (99%)	150/150 (100%)
Site #2 (%)	0/147 (0%)	82/148 (55%)	108/148 (73%)	136/149 (91%)	146/149 (98%)	150/150 (100%)
Site #3 (%)	0/149 (0%)	90/150 (60%)	139/150 (93%)	146/150 (97%)	150/150 (100%)	150/150 (100%)

Table 2: Reproducibility Results — Standard Specimen Processing Procedure

Results By Lot (# Positive / # Tested)						
	Negative	50 c/mL	100 c/mL	150 c/mL	250 c/mL	25,000 c/mL
Lot #1 (%)	0/90 (0%)	44/90 (49%)	75/89 (84%)	83/89 (93%)	85/88 (97%)	90/90 (100%)
Lot #2 (%)	0/89 (0%)	49/88 (56%)	72/88 (82%)	83/89 (93%)	86/89 (97%)	90/90 (100%)
Lot #3 (%)	0/89 (0%)	39/88 (44%)	72/89 (81%)	74/87 (85%)	86/90 (96%)	90/90 (100%)
Lot #4 (%)	1/87 (1%)	49/90 (54%)	59/88 (67%)	71/89 (80%)	85/90 (94%)	90/90 (100%)
Lot #5 (%)	0/89 (0%)	37/90 (41%)	65/89 (73%)	76/88 (86%)	85/89 (96%)	89/89 (100%)
Results By Site (# Positive / # Tested)						
Site #1 (%)	0/150 (0%)	73/149 (49%)	117/150 (78%)	134/150 (89%)	145/150 (97%)	150/150 (100%)
Site #2 (%)	0/144 (0%)	63/147 (43%)	109/144 (76%)	118/142 (83%)	138/146 (95%)	150/150 (100%)
Site #3 (%)	1/150 (1%)	82/150 (55%)	117/149 (79%)	135/150 (90%)	144/150 (96%)	149/149 (100%)

Analytical Sensitivity — Dilutional Panels

The analytical sensitivity of the COBAS AmpliScreen HIV-1 Test, v1.5 was determined by testing 10 HIV-1 seropositive clinical specimens. The titer of each specimen was quantitated with a commercially available assay using a secondary standard calibrated against the WHO International Standard. These specimens were diluted in normal human plasma to 150, 50, and 16.7 copies/mL for the Multiprep Specimen Processing Procedure and 300, 100, and 33.3 copies/mL for the Standard Specimen Processing Procedure.

The COBAS AmpliScreen HIV-1 Test, v1.5 detected 50 copies/mL HIV-1 RNA at a frequency greater than 98% with a lower 95% confidence limit of 96.5% using the Multiprep Specimen Processing Procedure. The assay detected 100 copies/mL HIV-1 RNA at a frequency greater than 98% with a lower 95% confidence limit of 96.5% using the Standard Specimen Processing Procedure. The data are presented in Tables 3 and 4.

When evaluated using PROBIT analysis, the combined data for all samples processed by the Multiprep Specimen Processing Procedure indicate an average 95% Limit of Detection (LOD) of 39.2 copies/mL, with the lower and upper 95% confidence limits of 34.0 copies/mL and 48.3 copies/mL, respectively. The LOD of 39.2 copies/mL corresponds to approximately 61.25 IU/mL.

When evaluated using PROBIT analysis, the combined data for all samples processed by the Standard Specimen Processing Procedure indicate an average 95% LOD of 96.2 copies/mL with the lower and upper 95% confidence limit of 83.3 copies/mL and 116.7 copies/mL, respectively. The LOD of 96.2 copies/mL corresponds to approximately 150.3 IU/mL.

Table 3: Multiprep Procedure Testing Summary for All Clinical Samples, Combined Input Values with 95% One-tailed Lower Confidence Limits

Multiprep Sample Processing Procedure				
HIV-1 RNA Concentration (c/mL)	Number of Positives	Number of Individual Trials	% Positive	95% Lower Confidence Limit (One-Tailed)
150	220	220	100.0%	98.6%
50	214	217	98.6%	96.5%
16.7	116	219	53.0%	47.2%

Table 4: Standard Procedure Testing Summary for All Clinical Samples, Combined Input Values with 95% One-tailed Lower Confidence Limit

Standard Sample Processing Procedure				
HIV-1 RNA Concentration (c/mL)	Number of Positives	Number of Individual Trials	% Positive	95% Lower Confidence Limit (One-Tailed)
300	216	218	99.1%	97.1%
100	216	219	98.6%	96.5%
33.3	97	217	44.7%	39.0%

Analytical Sensitivity — WHO HIV-1 International Standard

The analytical sensitivity of the COBAS AmpliScreen HIV-1 Test, v1.5 was also determined using the WHO HIV-1 International Standard (97/656). The WHO HIV-1 International Standard was serially diluted in HIV-1- negative plasma to final concentrations of 140, 100, 70, 50, 35, and 25 IU/mL for the Multiprep Specimen Processing Procedure and 800, 560, 400, 280, 200, and 140 IU/mL for the Standard Specimen Processing Procedure. Each dilution was tested using two lots of COBAS AmpliScreen HIV-1 Test, v1.5.

When evaluated using PROBIT analysis, the combined data from all samples using the Multiprep Sample Processing Procedure indicate an average 95% LOD of 78.4 IU/mL,

with lower and upper 95% confidence limits of 68.4 IU/mL and 94.4 IU/mL, respectively.

When evaluated using PROBIT analysis, the combined data from all samples tested using the Standard Sample Processing Procedure indicate an average 95% LOD of 323.4 IU/mL, with lower and upper 95% confidence limits of 284.9 IU/mL and 387.3 IU/mL, respectively.

Tables 5 and 6 summarize the overall results for the Multiprep and Standard Specimen Processing Procedures, respectively.

Table 5: Serial Dilution Testing Summary for Multiprep Method with HIV-1 RNA WHO International Standard (97/656) Combined Input Values with Lower 95% Confidence Limit (One-Sided)

HIV-1 RNA Concentration (IU/mL)	Number of Positives	Number of Individual Tests	% Positives	95% Lower Confidence Limit One-Sided
140	128	130	98.5%	95.2%
100	115	120	95.8%	91.4%
70	128	130	98.5%	95.2%
50	103	120	85.8%	79.5%
35	79	118	66.9%	59.1%
25	70	120	58.3%	50.4%

Table 6: Serial Dilution Testing Summary for Standard Method with HIV-1 RNA WHO International Standard (97/656) Combined Input Values with Lower 95% Confidence Limit (One-Sided)

HIV-1 RNA Concentration (IU/mL)	Number of Positives	Number of Individual Tests	% Positives	95% Lower Confidence Limit One-Sided
800	119	120	99.2%	96.1%
560	119	120	99.2%	96.1%
400	118	119	99.2%	96.1%
280	126	137	92.0%	87.1%
200	100	119	84.0%	77.5%
140	82	120	68.3%	60.6%

Analytical Sensitivity — CBER HIV-1 Panel

The FDA CBER HIV-1 Panel Members were processed using the Multiprep and Standard Specimen Processing Procedures. The Multiprep Specimen Processing Procedure detected 100% of all positive members ranging from 10 – 250,000 copies/mL. The Standard Specimen Processing Procedure detected 100% of all positive members ranging from 100 – 250,000 copies/mL. The data are shown in Table 7.

Table 7: FDA CBER HIV-1 RNA Panel Results

CBER HIV-1 (Copies/mL)	CBER HIV-1 Panel Test Results												
	A1 250,000	A2 25,000	A3 1,000	A4 100	A5 0	B1 2,500	B2 10	B3 250,000	B4 0	B5 100	B6 50	B7 25,000	B8 0
Multiprep Method	100%	100%	100%	100%	0%	100%	100%	100%	0%	100%	100%	100%	0%
Standard Prep Method	100%	100%	100%	100%	0%	100%	0%	100%	0%	100%	75%	100%	0%

Group / Subtype Detectability

One hundred culture specimens representing 20 each of HIV-1 Group M, subtypes A through E, 3 culture specimens of Subtype F, 4 culture specimens of Subtype G, 8 culture specimens of Group O, and 1 culture specimen of Group N were tested. The Group M specimens were tested at 400 copies/mL using the Standard Specimen Processing Procedure, and at 200 copies/mL using the Multiprep Specimen Processing Procedure. The Group O and N specimens were diluted 5-, 25-, 125-, 625-, and 3125-fold and tested using the Multiprep and Standard Specimen Processing Procedures. Data are provided in Table 8. Group O specimens were only evaluated as diluted samples due to limited specimen volume.

Table 8: HIV-1 Group/Subtype Tested

Group	Subtype	Quantity	Reactive Total (Multiprep)	Reactive Total (Standard Prep)
M	A	20	20/20	20/20
	B	20	20/20	20/20
	C	20	20/20	20/20
	D	20	20/20	20/20
	E	20	20/20	20/20
	F	3	3/3	3/3
	G	4	4/4	4/4
O*	N/A	8	5/8	5/8
N*	N/A	1	1/1	1/1

**Due to limited volume, specimens were only tested diluted and the actual HIV-1 RNA Group O and Group N copy numbers were not determined.*

Seroconversion Panels

Forty-one commercially available anti-HIV seroconversion panels were tested undiluted using the Standard Specimen Processing Procedure and diluted 1:24 using the Multiprep Specimen Processing Procedure. COBAS AmpliScreen HIV-1 Test, v1.5 detected HIV-1 RNA earlier than Abbott HIV 1/2 antibody test in 39 of the 41 panels, using both the Multiprep and Standard Specimen Processing Procedures.

COBAS AmpliScreen HIV-1 Test, v1.5 detected HIV-1 RNA a mean of 12.8 days (median 11 days, minimum 0 days and maximum of 89 days) before HIV 1/2 antibody using the Multiprep Specimen Processing procedure and a mean of 14.2 days (median 12 days, minimum 0 days and maximum of 89 days) before HIV 1/2 antibody when using the Standard Specimen Processing Procedure. The data are presented in Tables 9 and 10.

The COBAS AmpliScreen HIV-1 Test, v1.5 was also compared to the licensed HIV-1 p24 antigen assays (Abbott and Coulter). Forty of the 41 panels contained specimens collected before the antigenemia “ramp up” phase, and were used to assess the effectiveness of the COBAS AmpliScreen HIV-1 Test, v1.5 in closing the pre-seroconversion window period, as compared to licensed Abbott HIV-1 p24 antigen assays (due to limited volume, only 38 panels were tested with the licensed Coulter HIV-

1 p24 antigen test). In every instance where HIV-1 p24 antigen is detected, HIV-1 RNA was also detected in the same specimen time point. In some panels, HIV-1 RNA was detected before HIV-1 p24 antigen.

COBAS AmpliScreen HIV-1 Test, v1.5 detected HIV-1 RNA a mean of 4.4 to 6.8 days before the licensed HIV-1 p24 antigen tests using the Multiprep Specimen Processing procedure and a mean of 5.8 to 8.3 days before the licensed HIV-1 p24 antigen tests when using the Standard Specimen Processing Procedure. The data are presented in Tables 9 and 10.

Table 9: Summary of the Pre-Seroconversion Detection of HIV-1 RNA vs. HIV 1/2 Antibody and HIV-1 p24 Antigen Assays — Multiprep Specimen Processing Procedure

	Days before HIV – 1/2 Antibody (41 Panels Tested)	Days Before Abbott p24 Antigen (40 Panels Tested)	Days Before Coulter p24 Antigen (38 Panels Tested)
Mean	12.8	6.8	4.4
Median	11	5	3.5
Maximum	89*	32	28
Minimum	0	0	0

* For one panel, the time interval between sampling was 80 days.

Table 10: Summary of the Pre-Seroconversion Detection of HIV-1 RNA vs. HIV 1/2 Antibody and HIV-1 p24 Antigen Assays — Standard Specimen Processing Procedure

	Days before HIV – 1/2 Antibody (41 Panels Tested)	Days Before Abbott p24 Antigen (40 Panels Tested)	Days Before Coulter p24 Antigen (38 Panels Tested)
Mean	14.2	8.3	5.8
Median	12	7	5
Maximum	89*	32	28
Minimum	0	0	0

* For one panel, the time interval between sampling was 80 days.

Dilutional Sensitivity with Weakly Reactive HIV-1 p24 Antigen Samples

Twenty-five HIV-1 p24 antigen weakly positive (S/CO 1.00 to 3.7 using a licensed HIV-1 p24 EIA) samples were evaluated. These were diluted with HIV-1 negative plasma to 5,000 copies/mL and further diluted 1:24 to represent the Primary Pool. The HIV-1 RNA copy numbers were determined by a commercially available HIV-1 quantitative assay (Roche's AMPLICOR HIV-1 MONITOR™ Test). The final viral concentration was approximately 208 copies/mL. In addition, another set was diluted to 100 copies/mL. All 25 samples tested at 5,000 copies/mL were negative for HIV-1 p24 antigen. All 25 samples tested with COBAS AmpliScreen HIV-1 Test, v1.5 at the 1:24 dilution of the 5,000 copies/mL (208 copies/mL) and all 25 samples tested at 100 copies/mL were positive for HIV-1 RNA.

Dilutional Sensitivity with Weakly Reactive HIV-1 Antibody Positive Samples

Twenty-five known HIV-1 seropositive specimens were diluted to Signal/Cutoff (S/CO) levels between 1 and 5 and tested using a licensed HIV-1 antibody assay (Abbott HIVAB HIV-1/HIV-2 (rDNA) EIA). These weakly reactive, seropositive samples were then singly introduced into pools with 23 negative plasma samples in random fashion. An additional 144 negative plasma tubes were used to make six negative pools and randomly distributed as discrete sets among the 25 positive pools for testing. A total of 744 samples were tested according to the COBAS AmpliScreen test algorithm. NAT-positive specimens were deconstructed and resolved to the individual sample. Of the 25 weakly-reactive serologically positive samples, a total of 19 were concordant positive and six were discordant negative in the COBAS AmpliScreen HIV-1 Test, v1.5.

Each of the six discordant NAT-negative samples was subject to viral load determination by Roche's quantitative PCR assay, AMPLICOR HIV-1 MONITOR Test, v1.5. Five of

the six discordant NAT negative samples were observed to have less than 100 copies/mL HIV-1 RNA, and one had a mean titer of 100 copies/mL. Because each of these samples, when diluted 24-fold, would not be expected to be reliably detected in 24-membered mini-pools, they were removed from the sensitivity calculation. Therefore, the overall observed sensitivity of the COBAS AmpliScreen HIV-1 Test, v1.5, in this study was 100.0%.

Analytical Specificity — Potentially Cross Reactive and Interfering Microorganisms

The analytical specificity of the COBAS AmpliScreen HIV-1 Test, v1.5 was evaluated by testing a panel of microorganisms and other disease states, including 21 viral isolates, five bacterial strains and one yeast isolate. No-cross reactivity was observed with the COBAS AmpliScreen HIV-1 Test, v1.5. Table 11, below summarizes the microorganisms studied.

Table 11: Analytical Specificity — Microorganisms and Disease States Tested

Adenovirus type 2	Epstein Barr Virus	Human Papilloma Virus, Type 16
Adenovirus type 3	Hepatitis A Virus	Human Papilloma Virus, Type 18
Adenovirus type 7	Hepatitis B Virus	HTLV-I
Autoimmune samples	Hepatitis C Virus	HTLV-II
Burkitt's Lymphoma	Herpes Simplex type 1	<i>Neisseria gonorrhoeae</i>
<i>Candida albicans</i>	Herpes Simplex type 2	<i>Propionibacterium acnes</i>
<i>Chlamydia trachomatis</i>	HIV-2	<i>Staphylococcus aureus</i>
Coxsackievirus B1	Human Herpes Virus 6	<i>Staphylococcus epidermidis</i>
Cytomegalovirus	Human Herpes Virus 7	Varicella-Zoster
Echovirus 1, 5	Human Papilloma Virus, Type 6a	

Up to 25 individual patient plasma specimens from each of the following disease categories were spiked with low levels of HIV-1 positive plasma: HAV, HBV, HCV, HIV-2, autoimmune disease, EBV, CMV, and *Candida albicans*. No false negative test results were observed.

Analytical Specificity — Non-HIV-1 Samples

Up to 25 individual patient plasma specimens (all HIV-1 negative) from each of the following disease categories: HAV, HBV, HCV, HIV-2, autoimmune disease, EBV, CMV, and *Candida albicans*, were tested with COBAS AmpliScreen HIV-1 Test, v1.5 by using both Multiprep and Standard Specimen Processing Procedures. All samples were found to be negative. No false positive test results were observed.

Potentially Interfering Substances

Endogenous Interfering Substances

HIV-1 spiked and non-spiked plasma samples derived from whole blood containing abnormally high concentrations of bilirubin (up to 20 mg/mL), triglycerides (up to 3000 mg/dL), hemoglobin (up to 1.0 g/dL), and albumin (up to 6 g/dL) were tested. These endogenous substances did not interfere with the sensitivity or specificity of the COBAS AmpliScreen HIV-1 Test, v1.5 using either the Multiprep or Standard Specimen Processing Procedures.

Exogenous Interfering Substances

HIV-1 spiked and non-spiked plasma samples derived from whole blood containing abnormally high concentrations of aspirin (up to 50 mg/mL), pseudoephedrine-HCl (up to 3 mg/dL), ascorbic acid (up to 20 mg/dL), acetaminophen (up to 40 mg/dL), or ibuprofen (up to 40 mg/dL) were tested. These exogenous substances did not interfere with the sensitivity or specificity of the COBAS AmpliScreen HIV-1 Test, v1.5 using either the Multiprep or Standard Specimen Processing Procedures.

Clinical Performance

AIDS and HIV-1 Asymptomatic Populations

Seropositive samples from 217 patients diagnosed with AIDS and seropositive samples from 784 HIV-1 asymptomatic patients were randomly intermixed with 1,399 negative plasma samples. These 2,400 samples were used to create 100 Primary Pools that contained on average 10 positive and 14 negative samples. In addition, 600 negative samples were used to create 25 negative Primary Pools. This resulted in 125 panels, each representing a Primary Pool comprised of 24 sample tubes, (20 panels containing AIDS samples, 80 panels containing asymptomatic samples, and 25 negative panels). These panels were pooled using the Hamilton Microlab AT plus and tested with the COBAS AmpliScreen HIV-1 Test, v1.5. Primary, Secondary and Tertiary testing was performed at the clinical sites. If discordant results between the Primary Pool test result and either the Secondary or Tertiary testing were observed at the sites, resolution testing was performed at Roche.

A summary of the testing performed at the clinical sites is provided in Tables 12 and 13. There were a total of 23 HIV-1 antibody positive specimens that resulted in one or more HIV-1 RNA positive primary pools. All were found to be negative in either the secondary or tertiary testing at the clinical sites. Of these 23 specimens, 9 tested negative at the secondary pool level, in 5 different secondary pools at a single clinical site and 14 tested negative by tertiary testing. The results of the resolution testing performed at Roche yielded 21 of 23 specimens that were resolved as HIV-1 RNA positive with the COBAS AmpliScreen HIV-1 Test, v1.5. A summary of the testing after resolution at Roche is provided in Table 13. Sensitivity and specificity were based upon the final resolution status of all samples. Individual specimens known to contain less than 100 copies/mL were not included in the sensitivity calculation. The sensitivity of the COBAS AmpliScreen HIV-1 Test, v1.5 relative to antibody-negative status in this study was determined to be 99.7% with the 95% confidence interval ranging from 99.1% to 100%. The specificity of the COBAS AmpliScreen HIV-1 Test, v1.5 relative to antibody-

negative status in this study was determined to be 98.9% with the 95% confidence interval ranging from 98.3% to 99.3%.

Table 12: Results of HIV-1 Seropositive Specimens Tested at the Clinical Sites (Discordant Specimens < 100 Copies/mL Removed)

		HIV-1 Antibody		Total
		Positive	Negative	
COBAS AmpliScreen HIV-1 Test, v1.5 Result	Positive	733	22	755
	Negative	23	1977	2000
Total		756	1,999	2,755

Table 13: Results of HIV-1 Seropositive Specimens (Discordant Specimens < 100 Copies/mL Removed) Following Resolution Testing at Roche

		HIV-1 Antibody		Total
		Positive	Negative	
COBAS AmpliScreen HIV-1 Test, v1.5 Result	Positive	754	22	776
	Negative	2	1977	1979
Total		756	1,999	2,755

High Risk Population

Specimens were prospectively collected from a population of patients being evaluated at AIDS clinics. Specimens were tested in a blinded fashion in order to identify at least 50 HIV-1 RNA positives with the COBAS AmpliScreen HIV-1 Test, v1.5 using both the Standard Sample Processing Procedure and the Multiprep Sample Processing Procedure. Specimens for the Multiprep Procedure were diluted 1:24 with Normal Human Plasma. Samples for the Standard Procedure were tested without dilution.

Of 374 specimens tested, 55 were found positive for HIV-1 RNA when tested using the Standard procedure and 54 were found positive when tested using the Multiprep procedure. One sample was found to be positive when tested using the Standard Sample Processing Procedure but negative when diluted 1:24 with NHP and tested using the Multiprep Sample Processing Procedure. This sample was negative when tested by both p24 antigen and HIV-1 antibody tests, indicating that this sample may be a window period specimen. There were 54 of the 55 specimens that were confirmed positive for HIV-1 antibody by Western Blot. Samples were judged to be NAT/serology concordant if the NAT result was: 1) positive and at least one serologic assay is positive; or 2) negative and serologic assays are both negative. A total of 316 of the 374 samples were negative for HIV-1 antibody. There were three antibody-positive specimens that were negative for HIV-1 RNA using the COBAS AmpliScreen HIV-1 Test, v1.5. However, these specimens were negative by p24 antigen EIA, and when tested with a quantitative assay (AMPLICOR HIV-1 MONITOR Test, v1.5), the titer was below the assay detectable limit. The data are presented in Tables 14 and 15.

Table 14: Clinical Sensitivity in a High Risk Population with Standard Prep

Standard Prep		Total Tested		HIV-1 Antibody Reactive by EIA			HIV-1 Antibody Negative	HIV-1 Antibody Reactive by EIA, HIV-1 RNA [≥] 100 c/mL			HIV-1 Antibody Negative, RNA [≥] 100 c/mL
				Western Blot				Western Blot			
				NT	Neg / Ind	Pos		NT	Neg / Ind	Pos	
+	55	54	0	0	1	54	0	0	0		
-	319	0	7	3	309	0	0	0	307		

Table 15: Clinical Sensitivity in a High Risk Population with Multiprep

Multiprep		Total Tested		HIV-1 Antibody Reactive by EIA			HIV-1 Antibody Negative	HIV-1 Antibody Reactive by EIA, HIV-1 RNA [≥] 100 c/mL			HIV-1 Antibody Negative, RNA [≥] 100 c/mL
				Western Blot				Western Blot			
				NT	Neg / Ind	Pos		NT	Neg / Ind	Pos	
+	54	54	0	0	0	54	0	0	0		
-	320	0	7	3	310	0	0	0	307		

Pool Reactivity in Volunteer Blood Donors

A random selection of 10,727 primary pools revealed that 26 primary pools were reactive with the COBAS AmpliScreen HIV-1 Test, v1.5 for an initially reactive rate of 0.24%. There were 11 reactive pools with at least 1 confirmed anti-HIV positive specimen and 0 pools were positive due to confirmed window period cases. A total of 15 pools were reactive but were not confirmed. Results are summarized in Table 16.

Table 16: Pool Reactivity in Volunteer Blood Donors

Category	Pools	Percentage
Pools Tested	10,727	100
Non- reactive pools	10,701	99.75
Initially reactive pools	26	0.24
Initial pools with concordant positive serology	11	0.1
Positive pools due to window case	0	0
Initially reactive pools with negativeserology and negative individual donation AmpliScreen testing (false positive)	15	0.14

A total of 792,055 specimens were selected from geographically divergent sites. The results from these specimens were used to determine the specificity and sensitivity of COBAS AmpliScreen HIV-1 Test, v1.5. Using the antibody and antigen results, the HIV status of each specimen was determined. HIV status-negative included either: 1) anti-HIV EIA negative and HIV-1 p24 antigen negative (EIA nonreactive or neutralization negative) unless the subject was enrolled in the follow-up study and had test results that changed this assessment, or 2) anti-HIV EIA repeatedly reactive, WB/IFA negative and HIV-1 p24 antigen negative or indeterminate.

HIV status-positive included either: 1) anti-HIV EIA repeatedly reactive, WB/IFA positive, or 2) follow-up study test results of anti-HIV repeatedly reactive or HIV-1 RNA positive. HIV status unknown included anti-HIV EIA repeat reactive, WB/IFA indeterminate or unknown, HIV-1 p24 antigen negative or indeterminate.

There were 791,733 specimens that were determined to be HIV status-negative. Of these, 791,732 were also HIV-1 RNA-negative. The specificity of the COBAS AmpliScreen HIV-1 Test, v1.5 in this study was 791,732/791,733 or 99.9999% with 95% confidence limits of 99.99% to 100.00%.

There were 42 specimens that were determined as HIV status-positive. Of these, 38 were also HIV-1 RNA positive. The sensitivity of the COBAS AmpliScreen HIV-1 Test, v1.5 in this study was 38/42 or 90.48% with 95% confidence limits of 77.38% to 97.34%.

Detection of Window Period Cases

From November 11, 1999 to December 31, 2001, approximately 8 million donations were tested. During this period there were 2 confirmed window period cases detected. A confirmed window period case is defined as an enrolled individual from whom the index donation was positive in the COBAS AmpliScreen HIV-1 Test, v1.5 but non-reactive by EIA for HIV-1/2 and a follow-up specimen was shown to be anti-HIV EIA repeatedly reactive and/or HIV-1 RNA positive. The detection rate of such window period cases was 0.0000002% (1 in 4,000,000). There was one additional specimen that was anti-HIV EIA negative, HIV-1 p24 antigen positive and HIV-1 RNA positive; however, this donor was not enrolled in the follow-up study.

Performance of Single Donation Testing

A total of 587 specimens were tested individually in the COBAS AmpliScreen HIV-1 Test, v1.5 clinical trial. The HIV-1 status of these samples was based upon EIA and supplemental test results as described above.

Of the 587 specimens, 271 specimens had available HIV-1 antibody test data. Of these 271 specimens, 271 were classified as HIV-1 status-negative; there were no HIV-1 status- positive donors. The specificity of the COBAS AmpliScreen HIV-1 Test, 1.5 in this study was 100% (271/271) with 95% confidence interval of 98.65% to 100%. There were no HIV-1 RNA positive specimens detected by individual donation testing using the COBAS AmpliScreen HIV-1 Test, v1.5.

References

1. Barre-Sinoussi, F., Chermann, J.C., Rey, F. 1983. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immunodeficiency syndrome. *Science*. **220**:868-871.
2. Popovic, M., Sarngadharan, M.G., Read, E., et al. 1984. Detection, isolation and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. *Science*. **224**:497-500.
3. Gallo, R.C., Salahuddin, S.Z., Popovic, M., et al. 1984. Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at-risk for AIDS. *Science*. **224**:500-502.
4. Curran, J.W., Jaffe, H.W., Hardy, A.M., et al. 1988. Epidemiology of HIV-1 Infection and AIDS in the United States. *Science*. **239**:610-616.
5. Gaines, H., von Sydow, M.A., von Stedingk, L.V. 1990. Immunological changes in primary HIV-1 infection. *AIDS* **4**:995-999.
6. Tindall, B., and Cooper, D.A. 1991. Primary HIV-1 infection: host responses and intervention strategies. *AIDS* **5**:1-14.
7. Daar, E.S., Moudgil, T., Meyer, R.D., et al. 1991. Transient high levels of viremia in patients with primary human immunodeficiency virus type 1 infection. *New England Journal of Medicine* **324**:961-964.
8. Clark, S.J., Saag, M.S., Decker, W.D. 1991. High titers of cytopathic virus in plasma of patients with symptomatic primary HIV-1 infection. *New England Journal of Medicine* **324**:954-960.
9. Albert, J., Abrahamsson, B., Nagy, K. 1990. Rapid development of isolate-specific neutralizing antibodies after primary HIV-1 infection and consequent emergence of virus variants which resist neutralization by autologous sera. *AIDS* **4**:107-112.

10. Hornsburgh, C.R., Jason, J., Longini, I.M., et al. 1989. Duration of human immunodeficiency virus infection before detection of antibody. *Lancet* **26**:637-640.
11. AIDS epidemic update-December 1998. UNAIDS Joint United Nations Programme on HIV-1/AIDS.
12. Dodd, R.Y. 1994 Adverse consequences of blood transfusion: quantitative risk estimates. In: Nance ST, ed. *Blood supply: risks, perceptions and prospects for the future*. Bethesda: American Association of Blood Banks 1-24.
13. Schreiber GB, Busch MP, Kleinman SH, Korelitz JJ. 1996 The risk of transfusion-transmitted viral infections. The Retrovirus Epidemiology Donor Study. *New England Journal of Medicine* **334**:1685-90.
14. Holland PV. 1996 Viral infections and the blood supply (editorial). *New England Journal of Medicine* **334**:1734-35.
15. Kleinman SH, Busch MP. General overview of transfusion transmitted infections. In: Petz LD, Swisher S, Kleinman SH, Spence R, Strauss RG, eds. *1995 Clinical practice of transfusion medicine*, 3rd ed. New York: Churchill Livingstone 809-21.
16. Busch MP, Stramer SL, Kleinman SH. 1997 Evolving applications of nucleic acid amplification assays for prevention of virus transmission by blood components and derivatives. In: Garratty G, ed. *Applications of molecular biology*. Bethesda, American Association of Blood Banks 121-73. (Presented at a workshop during the 50th Annual Meeting of the AABB, October 1997, Denver, CO).
17. Busch MP, Lee LL, Satten FA, et al. 1995 Time course of detection of viral and serologic markers preceding human immunodeficiency virus type 1 seroconversion: implications for screening of blood and tissue donors. *Transfusion* **35**:91-7.
18. Henrard DR, Phillips J, Windsor I, et al. Detection of human immunodeficiency virus type 1 p24 antigen and plasma RNA: relevance to indeterminate serologic test. *Transfusion* 1994;**34**:376-80.

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19. Soriano V, Dronda F, Gonzalez-Lopez A et al. 1994 HIV-1 causing AIDS and death in a seronegative individual [letter]. *Vox Sang* **67**:410-11.
 20. Centers for Disease Control and Prevention. Persistent lack of detectable HIV-1 antibody in a person with HIV-1 infection - Utah, 1995. *MMWR* 1996 **45**:181-85.
 21. Saiki, R.K. Scharf, S., Faloona, F., et al. 1985. Enzymatic amplification of β -Globin genomic sequences and restriction site analysis for diagnosis of Sickle Cell Anemia. *Science*. **230**:1350-1354.
 22. Saiki, R.K., Gelfand, D.H., Stoffel, S., et al. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science*. **239**:487-491.
 23. Mullis, K.B., Faloona, F.A. 1987. Specific synthesis of DNA *in vitro* via a polymerase-catalyzed chain reaction. *Methods in Enzymology*. **155**:335-350.
 24. Mulder, J. McKinney, N., Christopherson, C., et al. 1994. Rapid and simple PCR assay for quantitation of human immunodeficiency virus type 1 RNA in plasma: Application to acute retroviral infection. *Journal of Clinical Microbiology*. **32**:292-300.
 25. Mortimer J. 1997 Intersecting pools and their potential application in testing donated blood for viral genomes. *Vox Sanguinis* **73**:93-6.
 26. Yerly, S., Pedrocchi, M., and Perrin, L. 1998. The use of polymerase chain reaction in plasma pools for the concomitant detection of hepatitis C and HIV-1 type 1 RNA. *Transfusion*. **10**:908-914.
 27. Myers, T.W., and Gelfand, D.H. 1991. Reverse transcription and DNA amplification by a *Thermus thermophilus* DNA polymerase. *Biochemistry*. **30**:7661-7666
 28. Kwok, S., Sninsky, J.J. 1993. PCR detection of human immunodeficiency virus type 1 proviral DNA sequences. In: *Diagnostic Molecular Biology: Principles and Applications*. Eds. Persing, D.H., Smith, T.F., Smith, F.C., et al. ASM, Washington, D.C.

-
29. Longo, M.C., Berninger, M.S., Hartley, J.L. 1990. Use of uracil DNA glycosylase to control carry-over contamination in polymerase chain reactions. *Gene*. **93**:125-128.
 30. Richmond, J.Y. and McKinney, R.W. (eds.). 1993 *Biosafety in Microbiological and Biomedical Laboratories* HHS Publication Number (CDC) 93-8395.
 31. National Committee for Clinical Laboratory Standards. 1997. Protection of Laboratory Workers from Infectious Disease Transmitted by Blood, Body Fluids, and Tissue. Approved Guideline. NCCLS Document M29-A Villanova, PA:NCCLS.
 32. International Air Transport Association Dangerous Goods Regulations, 41st Edition. 2000. 704 pp.

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